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# The spectrum of intermediate *SCN8A*-related epilepsy

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**Abstract:**

Objective: Pathogenic variants in *SCN8A* have been associated with a wide spectrum of epilepsy phenotypes, ranging from benign familial infantile seizures (BFIS) to epileptic encephalopathies with variable severity. Furthermore, a few patients with intellectual disability (ID) or movement disorders without epilepsy have been reported. The vast majority of the published *SCN8A* patients suffers from severe developmental and epileptic encephalopathy (DEE). In this study, we aimed to provide further insight on the spectrum of milder *SCN8A*-related epilepsies.

Methods: A cohort of 1095 patients was screened using a next-generation sequencing panel. Further patients were ascertained from a network of epilepsy genetics clinics. Patients with severe DEE and BFIS were excluded from the study.

Results: We found 36 probands who presented with a *SCN8A*-related epilepsy and normal intellect (33%) or mild (61%) to moderate ID (6%). All patients presented with epilepsy between age 1.5 months and seven years (mean 13.6 months), and 58% of these became seizure free, 2/3 on monotherapy. Neurological disturbances included ataxia (28%) and hypotonia (19%) as the most prominent features. Interictal EEG was normal in 41%. Several recurrent variants were observed including Ile763Val, Val891Met, Gly1475Arg, Gly1483Lys, Phe1588Leu, Arg1617Gln, Ala1650Val/Thr, Arg1872Gln and Asn1877Ser.
Significance: With this study, we explore the electro-clinical features of an intermediate *SCN8A*-related epilepsy with mild cognitive impairment and for the majority a treatable epilepsy.

**Key words**: *SCN8A*, voltage-gated sodium channels, epilepsy, epilepsy genetics, intellectual disability

**Introduction:**

*SCN8A* encodes the voltage-gated sodium channel $Na_v1.6$, which is primarily expressed in excitatory neurons with high concentrations at the axon initial segment and the node of Ranvier. Pathogenic variants in *SCN8A* are associated with a spectrum of epilepsy phenotypes, ranging from rare families with benign familial infantile seizures (BFIS) to severe early onset developmental and epileptic encephalopathies (DEE) (EIEE13, OMIM #614558). Since the first case report published by Veeramah and colleagues in 2012[1], several reports have confirmed the role of *SCN8A,* primarily in patients with DEE[2-9]. The majority of patients with *SCN8A* DEE, described so far, have a severe phenotype characterized by early seizure onset, difficult to treat seizures, severe intellectual disability (ID), motor disorders and a relatively high mortality[2-10].
Functional studies of selected variants causing DEE, have revealed gain-of-function as the main pathogenic mechanism[1; 6; 11; 12]. This gain-of-function comes from hyperactivity of the ion channel, due to elevated persistent sodium currents, hyperpolarizing shifts in the voltage dependence of activation or impaired channel current inactivation[12; 13]. This mechanism is the opposite of the one that has been demonstrated in Dravet syndrome, which is characterized by loss-of-function variants in *SCN1A*[14]. *SCN1A* encodes the voltage gated sodium channel Nav1.1, which is primarily expressed in inhibitory interneurons. Variants in *SCN2A*, encoding a third voltage-gated sodium

channel in the human CNS, can lead to both gain- and loss-of-function, complicating things even more[15]. Awareness of these important differences in pathophysiology is necessary, as it might have therapeutic implications[16].

We have recently detected a recurrent *SCN8A* variant, Glu1483Lys, in a very mild familial epilepsy phenotype[17]. We identified three unrelated families with a total of 16 family members who presented with BFIS and normal cognition. Five family members developed paroxysmal kinesigenic dyskinesia (PKD)[17]. Recently, Han et al. confirmed BFIS as part of the phenotypic spectrum in *SCN8A*-related epilepsies[18]. Beside these two extreme phenotypes, there is an increasing number of patients with a milder form of DEE. In this study, we describe the spectrum of the electro-clinical features of this intermediate *SCN8A*- epilepsy phenotype, aiming to provide a clearer picture for clinicians, genetic counselors and affected families.

**Methods and material:**

We systematically screened all exons and exon-intron boundaries of *SCN8A* in a cohort of 1095 unselected patients with various forms of epilepsy using different next-generation sequencing (NGS) panels[19]. The panels included from 45 to 500+ genes related to epilepsy, intellectual disability or autism. Variants were assumed to be pathogenic if they arose *de novo*, or were inherited from an affected parent or affected/unaffected mosaic parent, and if they were non-synonymous, splice-site altering or frameshift causing, and not present in controls in the gnomAD browser (see web resources). Sanger sequencing confirmed variants and segregation. Furthermore, detected variants were tested (PolyPhen-2, SIFT and MutationTaster) for predicted pathogenicity. ACMG and MPC scores are noted in table 1. All variants, except for one, were either pathogenic or likely pathogenic according to the ACMG criteria[20].

Four different metrics were used for in silico variant pathogenicity prediction:

1) The 'Missense badness, PolyPhen-2, and Constraint score' (MPC), which demonstrates a 5.8 times increased variant enrichment in cases compared to individuals from the general population for MPC scores >2[21]

2) The 'paralog conservation score' (parazscore), which quantifies the amino-acid positions conservation across human proteins of the same gene family. A significant enrichment of disease-associated missense variants was observed at paralog-conserved sites[22];

3) The Grantham score, which accesses the effect of the amino acid substitution based on the properties of the amino acid exchange. It ranks amino acid substitutions from similar amino acids substitiutions (0) to substitution which differs in their chemical properties ones (215)[23].
4) The allele frequency analysis based on the alleleFrequencyApp (see web resources), which calculates a maximum credible number of possible pathogenic alleles observed in gnomAD. The allele count is estimated based on the disease prevalence, the allelic and genetic heterogeneity and the variant penetrance. For *SCN8A* we specified the disease prevalence as one in 300 the allelic heterogeneity as 0.01, the genetic heterogeneity as 0.1 and the penetrance as 50% resulting in a maximum number of a single allele in gnomAD. We compared the corresponding value with the allele frequency of variants present in gnomAD.

*SCN8A* positive patients underwent a detailed clinical evaluation and patients with severe DEE and BFIS were excluded from the study. The criteria for severe DEE were defined as severe, pharmacoresistant epilepsy and developmental impairment, as previously reported [24]. BFIS is a self-limiting epilepsy syndrome characterized by afebrile seizures typically in clusters, with onset between four and eight months. Neurological examination, psychomotor development and the interictal EEG are normal and the children become seizure free within the first years of life and sustain seizure freedom without the aid of antiepileptic drugs (AEDs)[25].
Additional probands were collected from an international network of epilepsy genetics clinics. Data on clinical phenotype, genetics, neuroimaging and EEG were requested for all patients (and if possible relatives), who were included in the study. Seizures were classified according to the International League Against Epilepsy (ILAE)[26]. All probands, and in case of minors, legal parents, provided written informed consent. The study was approved by the local ethical committees.
Sodium channel blockers (SCBs) were defined as AEDs that target sodium channels and included carbamazepine (CBZ), oxcarbazepine(OXC), lamotrigine(LTG) and phenytoin (PHT).
Data sharing: anonymized data will be shared by request from any qualified investigator.

**Results:**

By the use of targeted NGS screening of *SCN8A* in a cohort of 1095 patients, we identified 12 (1.2%) probands with a predicted pathogenic variant in *SCN8A*. Six of the patients fulfilled the

criteria for a severe DEE and thus excluded from this study. Of the 1095 patients screened, approximately 326 fulfilled a DEE diagnosis (this might be an underestimate, as referrals to our center are often lacking clinical information). In addition to the remaining six patients, we ascertained 30 additional probands/families with predicted pathogenic *SCN8A* variants through collaborating diagnostic and research laboratories. The Asn1877Ser variant was seen in three controls in gnomAD, but as the phenotype resembles a benign familial epilepsy, with several family members affected, and was seen in three probands in this study, it was included as being pathogenic. All variants, except #10 p.Tyr1241Cys, were classified as pathogenic or likely pathogenic according to the ACMG criteria[20]. Not all missense variants in *SCN8A* are pathogenic and at 384 amino acid positions variants have been reported in the general population[27]. Distinguishing disease-causing variants from benign is still a challenge in clinical genetics. Rarity of an allele is widely recognized as a necessary (though not sufficient) criterion for variant pathogenicity[20] (ACMG guidelines, but the key question "how common is too common?" remains poorly answered for many diseases.) Estimating the genetic and allelic heterogeneity offers an opportunity to identify variant cut-off frequency filters. Genetic heterogeneity is the maximum proportion of disease that is attributable to variation in a single gene, and allelic heterogeneity is the maximum proportion of variation within a gene that is attributable to a single allele.
The patient variant 10 (c.3722A>G, p.Tyr1241Cys, NM_014191.3) was found once in the gnomAD[27] and discovEHR[28] database. The presence of a variant in databases represents not a pathogenicity exclusion criterion based on our allele frequency analysis for mild forms of epilepsy. The amino acid residue position is relatively conserved across voltage-gated sodium channels (positive parazscore of 0.23[22]). In addition, the MPC score (MPC = 2.48) supports variant pathogenicity and the Grantham score of 194 indicates a likely pathogenic variant.
One of the probands (#30) has previously been mentioned briefly[29], but was included in this study since additional data had been collected.
In total, 36 probands/families were included. The detected *SCN8A* variants were mainly missense variants (33/36) scattered throughout the gene. In addition, two truncating variants (one frameshift (#4) and one stop (#30)), and one variant causing a nucleotide change eight basepairs upstream of the start codon (#1) were detected. Fourteen variants were located in the transmembrane domains and two in the poreforming domain, of the cytoplasmic variants, we

found one in the D1/D2 domain. The variant Asn544fs* was a frameshift variant with a clinical picture of absence epilepsy, inherited from an affected parent, and classified pathogenic according to the ACMG classification guidelines.

The variants either occurred *de novo* or segregated within the family in a dominant fashion (#2, #4, #7, #14, #15, #33 and #34, see figure 1 for selected pedigrees). In the family of proband #33 the variant was inherited from an affected mosaic parent. Mosaicism was also suspected in #15, but has not yet been confirmed. All variants were located at highly conserved residues and predicted possibly damaging according to computational prediction software (see methods and web resources). Mining the available literature and databases, eight variants were found to be recurrent either within this study or overall; Ile763Val[30], Val891Met[31], Gly1475Arg[10; 32], Gly1483Lys[17], Phe1588Leu, Arg1617Gln[5; 7; 8; 33; 34], Ala1650Val[5; 7; 35] (different amino acid substitution, see discussion section), Arg1872Gln[7; 12; 36] and Asn1877 Ser[30; 35; 37], with five of them (amino acid positions 763, 1475, 1617,1650, 1872) seen in severe DEE phenotypes as well.

Clinical data on all 36 probands and families are presented in table 1. Age at inclusion varied from nine months to 35 years, with a mean of 7.9 years. Seizure onset was between six weeks and seven years, with a mean of 13.6 months (SD ±17 m). Seizure semiology was very diverse, and included generalized tonic-clonic seizures (GTCs) (21), focal (14), tonic (8), myoclonic (6) and atonic seizures (8), as well as epileptic spams (2) and atypical / typical absences (12). Seizure severity did not progress over time and seizure triggers were not found. Three probands experienced convulsive status epilepticus (#3, #14, #33).

The interictal EEG was available in 33/36 patients and showed focal epileptiform abnormalities in 15 patients (45%), predominant in the posterior quadrants in (ten patients) or in the central/ centro-parietal / fronto-central regions (five patients), with or without bilateral spreading. Four patients (12%) had only generalized abnormalities (#6, #14, #22, #26). In 14 patients (39%) the interictal EEG was normal (#9, #11, #12, #13, #16, #17, #20, #23, #29, #34, #35) or normalized at follow up (#15). Ictal EEG was available in four patients and showed a focal discharge in two (#6, #11) and generalized spike or irregular spike and waves discharges in the other two (#19, #28).

Cognitively, these patients fare well. Before seizure onset 26/36 (72%) had normal cognitive development, two/36 (6%) had a mild ID, one/36 had moderate ID (3%) and four/36 (11%) had developmental delay, not classified due to the young age of the patient. At follow-up, a

deterioration from normal intellect/developmental delay to mild ID or from mild ID/developmental delay to moderate ID was seen in seven (19%) patients, whereas 81% did not experience deterioration at seizure onset. After seizure onset (and at the time of follow-up) 22/36 (61%) had mild ID and two/36 (6%) had moderate ID.

Additional features included ataxia (10/36), hypotonia (7/36), language delay (5/36), autism/autistic features (4/36), movement disorders (3/36) including paroxysmal dyskinesia (3/36), gait disturbances (2/36), sleep disturbances (1/36), learning difficulties (2/36) and ADHD (1/36). See table 1 for details.

All probands were evaluated for their treatment response. Twenty-one/36 (58%) probands became seizure free. Monotherapy was sufficient in 12/21 (57%) probands, and included lamotrigine (LTG) (2), carbamazepine (CBZ) (1), phenytoin (PHT) (1), valproate (VPA) (1), vigabatrin (VGB) (1), ethosuximide (ETX) (1), phenobarbital (PB) (1) and levetiracetam (LEV) (1). In total 13/36 (36%) probands became seizure free with therapy that included a SCB either in monotherapy or in combination with other antiepileptic drugs (AEDs). One became seizure free with a small dose of cannabidiol. However, 7/36 (20%) probands became seizure free without the use of SCBs. Seizure offset was only available for six patients, and ranged between four and 10 years, mean age at seizure offset was 7.7 years.

Six probands had affected family members, segregating with the variants. For proband #5 a similar phenotype with absences and learning/language difficulties was seen in the sister, as well as the mother. For proband #7, the mother also had unspecified epilepsy and carried the variant; there was also an affected maternal grandfather, who did not have genetic analysis done. Likewise, proband #14 had an affected father, who carried the variant, and an affected paternal grandfather, who was not tested genetically. Proband #15 had a sister with a similar epilepsy phenotype, the variant was suspected to have been inherited from a mosaic parents, but this was not confirmed at the time of preparation of this paper. Proband #33 had an affected sister with a similar phenotype, and genetic investigations showed that the variant was inherited from an affected mosaic (28%) father. Proband #34 had an affected sister and mother, in whom the variant segregated.

**Discussion:**

Pathogenic variants in *SCN8A* have so far been described in patients with different epilepsy phenotypes, including rare families with BFIS and in >100 patients with mild to severe DEE. In this study, we describe the electro-clinical phenotype of 36 patients with intermediate epilepsies due to pathogenic variants in *SCN8A.* Patients with *SCN8A*-related severe DEE[21] and BFIS[18] were excluded from the study.

The variant at position c.-8A>G was assumed to be likely pathogenic because it occurred de novo and the clinical features of the patient resembled the phenotype seen in other SCN8A patients. The variant is located outside of the Kozak consensus sequence, but may lead to increased RNA stability or translational initiation or result in altered splicing pattern. These theories can only be confirmed by functional testing of the variant, which unfortunately was not possible in this study. Until then, the variant may need to be classified as a variant of unknown significance.

All 36 patients presented with seizures in early childhood (mean 13.6 months). Before seizure onset, 72% probands had normal cognitive development. More than half of the probands became seizure free, 57% of these with monotherapy. Compared to the BFIS families, described by Gardella et al[17], Anand et al[37] and Han et al[18], the majority of the probands in this cohort have cognitive impairment, with 6% suffering from moderate ID, and 61% from mild ID. Furthermore, only 58% became seizure free compared to almost 100% of the BFIS patients; seizure freedom is exceptional in the severe DEE phenotype. The patients herein described also appeared to have additional neurological disturbances including primarily ataxia (in 28%) and hypotonia (in 19%). In the severe DEE cohort, the incidence of ataxia is around 11%, compared to the 28% of this cohort. However, it is important to notice that many of the patients suffering from severe *SCN8A* DEE are unable to walk autonomously. Furthermore, the patients in this intermediate cohort do not suffer from the spasticity and paraplegia or the extra-pyramidal/cerebellar symptoms that up to 50% of the severe DEE patients do[24]. A few patients in this cohort (8%) had dyskinesia, which is also seen in both severe DEEs and BFIS families. Growth impairment (microcephaly or reduced growth) observed in severe DEE, was not seen in this cohort. Other prominent phenotypic features included language delay/difficulties in 14% and movement disorders not further specified in 8%.

In mouse models, it has been shown that *SCN8A* is widely expressed, both in the motor neurons of the brain stem, as well as in many types of neurons in the cerebellum, where functional deficits in Purkinje cells have been found[38-40], confirming the importance of *SCN8A* in motor function. This

could explain the involvement of the motor system, and why cerebellar atrophy and ataxia[41], associated with intellectual impairment, appeared to be major features in subjects harboring *SCN8A* variants. The cerebellum does, however, also play an important role in language and grammar processing, verbal working memory and speech motor planning[42], and a large proportion of *SCN8A* patients, including those reported in the present cohort, show an impairment of these functions as well.

When comparing the present cohort to the severe *SCN8A* DEE population, in which the patients have earlier seizure onset, with more pronounced cognitive deficits as well as refractory epilepsy, we tried to identify possible predictive factors, that in newly diagnosed patients could help to detect those with a milder course as compared to those with a more severe evolution. First, within our cohort, the majority of probands have normal development prior to seizure onset (and 33% of them continue to develop normally) and maintain a normal EEG (41%). This is often not the case in probands who develop severe DEE, in which the majority usually are cognitively delayed from birth[43], or will present with cognitive difficulties early on and show changes in their EEGs. However, it is not an absolute feature, as we have observed several children with an extremely severe follow up, despite early normal development. Second, seizure onset occurred at a mean age of 13.6 months, compared to a mean age of 4 months in the DEE group[43]. Anyway, it is worth to bear in mind that the age deviation in this cohort is quite large (SD±17 months), and thus these numbers should be interpreted carefully when counselling a family. Last, seizure freedom was obtained in 58% of the patients in this cohort and it was achieved rapidly and with monotherapy in 2/3. In contrast, only about 5% of patients in the severe DEE cohort achieve seizure freedom with monotherapy. This is important, albeit also a prerequisite in this study, where the more severe epilepsies were excluded.

Previously, it has been hypothesized, that seizures in patients with *SCN8A* variants should respond to SCBs [16; 17]. A beneficial effect of SCBs was observed in 36% of the patients reported in this study, either as monotherapy or in combination with other AEDs, and supportive of this, previous studies have shown a gain-of-function of the Arg1617Gln and Arg1872Leu[12] variants. Yet, 19% became seizure free without the use of SCBs, suggesting that seizure freedom should not be attributed solely to the use of SCBs, but also considered a phenotypic trait.

Interestingly, a partial benefit of LTG was also observed in the proband with a stop variant (#30). We can speculate that this unexpected finding may depend on genetic modifiers and differences in genetic background, or the fact that as the stop-codon is located at the c-terminal part of the protein, the transcript does not undergo nonsense-mediated decay.

Furthermore, we found three variants, i.e. one eight basepairs upstream of the start codon (#1), one frameshift variant (#4) and one stop variant (#30), all suspected to be loss-of-function, and all of them associated with an epilepsy phenotype. Previously, it has been hypothesized, that loss-of-function variants would not cause epilepsy[44], but rather present with ID with or without motor function abnormalities, such as ataxia[2; 34; 41]. However, Blanchard et al. also describe a patient with epilepsy and ID, carrying a LOF variant[11], and two existing *Scn8a* knock-out mice models, supports the notion of epilepsy also being a feature of LOF of NaV1.6[45; 46]. Other ion channel genes, including *SCN2A* have been shown to have a similar clinical picture, where both GOF and LOF variants may cause epilepsy. The underlying functional causes are yet to be fully elucidated.

Variants were found throughout the gene (see figure 3), including the transmembrane segments, cytoplasmic loops and the inactivation gate, thus location in the gene is likely not predictable of functional or clinical effects of the variant, however, the figure does display the fact that the majority of the variants are found in domain three and four, the inactivation gate and the c-terminal, which may help guide variant interpretation.

Indeed, we identified several recurrent variants and observed a wide range of phenotypic variability for variant carriers. Ile763Val, Gly1475Arg, Arg1617Gln, Ala1650Val and Arg1872Gln were all seen in this study as well as in patients with DEE. Ile763Val has previously been described in a patient with intractable epilepsy and moderate ID[30], whereas we found it in two patients with focal epilepsy and mild ID (#5 and #6).

The Gly1475Arg variant is even more diverse and it has previously been identified in several patients, including a child with severe DEE, who died from probable SUDEP[10; 32]. In this cohort, we found it in two patients with mild ID, and epilepsy controlled by SCBs. Both patients did suffer from hypotonia and ataxia.

Arg1617Gln has been seen in several DEE patients previously, including a girl with severe DEE who died after terminal progression of her disease[2, 17], whereas we found it in two patients with mild ID and treatable epilepsy (#24, #25). Still, they did display additional neurological disturbances

(language delay, dyskinesia) and their seizure onset was earlier (4 months), compared to the rest of this cohort. We also found the Arg1617Gln variant in one patient (#21), who displayed moderate ID as well as autism. Ala1650Val variant has not been described before (#26), but has been seen several times with a threonine (Ala1650Thr) substitution in patients with severe DEE[5; 7; 35]. Arg1872Gln has also been seen several times in patients with severe DEE[7; 36], however we found it in a sib pair, with normal intellect and focal epilepsy (#34). The Gly1483Lys variant has previously been described in BFIS[17]; the patient in this study (#18) carrying this variant has indeed a very mild phenotype; with only speech delay, sporadic seizures and discrete focal EEG abnormalities. Why some variants show phenotypic heterogeneity and other variants do not, remains elusive. Of course, differences in the amino acid substitution might explain some of the difference, but this is true for just a few of the variants. In other cases, genetic modifiers and differences in the genetic background could underlie these observations. Further studies are needed to investigate this further.

In conclusion, this study we provide further insight on the phenotypic spectrum of *SCN8A* epilepsy by focusing an intermediate phenotype characterized by treatable epilepsy with a later age of onset, mildly impaired cognitive development, as well as variable but in general mild neurological disturbances. A positive response to epilepsy treatment, especially with SCBs, was observed. Even if a wide range of phenotypes related to *SCN8A* variants can be expected (see illustration in figure 2), these findings highlight the presence of an increasing number of *SCN8A* patients with a phenotype of moderate severity.

The partial overlapping of genetic and early clinical features in *SCN8A*-related epilepsies makes it difficult to provide proper counselling in these children so far. Further investigations are warranted to clarify this issue, as well as explore possible prognostic factors.

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**Conflicts of interest:**

None of the other authors have any conflict of interest to disclose.

**Ethical publication statement:**

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**Web resources:**

- The ExAC browser: http://exac.broadinstitute.org
- The GnomAD database: http://gnomad.broadinstitute.org/
- SIFT: http://sift.jcvi.org
- Poly-Phen2: http://genetics.bwh.harvard.edu/pph2/
- MutationTaster: http://www.mutationtaster.org
- The allele frequency app: https://www.cardiodb.org/allelefrequencyapp

**Key Bullets:**

- The phenotypic spectrum of *SCN8A* is wide, from BFIS to DEE
- The intermediate phenotype is characterized by a treatable epilepsy and mild cognitive delay
- Prognostic markers remain elusive

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**Table 1. Clinical characteristics of the probands**

| Proband # | Age at inclusion | Variant, Inheritance, location | Pathogenicity (ACMG guidelines, MPC) | Family history | Age at seizure onset | Seizure types | Interictal EEG / Ictal EEG | Cognition before sz onset / cognition after sz onset | Treatment response | Succesfull monotherapy | Other features |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 y 8 m | c.-8A>G *de novo* 5'UTR | Pathogenic | None | 11 m | GTC, AA | EDs on the P-temporal regions bilaterally, with rare diffuse spreading | N/ MID | Sz reduction: VPA 50% | | Hypotonia, uncoordinated movements, gait disturbance |
| 2 | 9 y | c.411C>G p.Ile137Met *maternal* transmembrane domain D1S1 | Likely pathogenic (PM1, PM2, PP1, PP2, PP3) MPC=1.66 | Mother, at 10-11 y: episodes of loss of balance, lower limb hypostenia and falls, gait disturbance and hand tremor | 2 y 2 m | Staring/ hypotonia to GTC, A, F (visual) | Temporal-O spikes / SW (left side predominance) | MID/MID | Sz reduction: LTG, VPA, ZNS No effect: LEV, RFN, TPM | | Hypotonia, Hypothyroidism Enuresis, sleep disorders |
| 3 | 9 y 4 m | c.1122C>G p.Asn374Lys *de novo* poreforming | Likely pathogenic (PS2, PP2, PP3) | maternal cousin with sz | 7 y | Nocturnal frontal, T, GTC, SE | Multifocal SW, paroxysmal beta activity that lasts | N / MID | Sz free: PHT, VPA No effect: OXC, LEV, | | Gait disturbance |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | | domain | MPC=1.83 | | | | between 1-2 minutes | | ZNS, CLB | | |
| 4 | | c.1630_1631del p.Asn544fs*39 *maternal* cytoplasmic domain D1/D2 | Pathogenic (PVS1, PM2, PP1) | See fig 1. | 2 y 9 m | Ab | Slow wave and SW discharges | N / MID | Sz free: VPA + ESM+ AMD Sz reduction: VPA + ESM | | |
| 5 | 13 y | 2287A>G p.Ile763Val *de novo* transmembrane domain D2S1 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=1.51 | Cousin with febrile sz | 7 wk | GTC | NA | NA / MID | Sz free: CLB, ZNS, PHT, LTG, CBZ Adverse effects: LEV (behavior), CLZ and LZP (sleepiness) | | Hypotonia, ataxia, chronic constipation, premature adrenarche, periodic leg movement |
| 6 | 20 y | c.2287A>G p.Ile763Val *de novo* transmembrane domain D2S1 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=1.51 | None | 3 m | S, F | EDs, both hemispheres Ictal: right F-temporal onset, then rhythmic slow activity propagating | NA / MID | No effect: VPA, CBZ, LTG, CLB, acetazolamide | | Ataxia |

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| | | | | | | | into the right parasaggital regions | | | | |
| 7 | 11 y | c.2671G>A p.Val891Met *maternal* transmembrane domain D2S5 | Pathogenic (PS1, PM1, PM2, PP1, PP2) MPC=3.14 | See fig. 1 | 3 y | GTC, AA | CP and midline spikes | N / MID | Sz free: CBZ | CBZ | ADHD |
| 8 | 10 y | c.2806G>A p.Glu936Lys *non-maternal* poreforming domain | Likely pathogenic (PM1, PM2, PP1, PP2) MPC=3.33 | Father with ID and ADHD | 5 y | F, AA, FS | SW left F, C, right P | NA / MD | Sz free: LEV Adverse effects: TPM | LEV | Autistic features, behavioral problems |
| 9 | 9 y 9 m | c.3601G>A p.Glu1201Lys *de novo* transmembrane domain D3S1 | Likely pathogenic (PS2, PM1, PM2, PP2) MPC=2.37 | None | 8 m | M | Initially normal (1y - 3y4 m), then BG slowing (7-10y) Postictal: abundant beta, diffusely slow BG | DD / MID | Sz reduction: VPA, LEV, CLB No effect: STP Sz aggravation. OXC | | Hypotonia, ataxia, paroxysmal dystonia |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 2 y | c.3722A>G<br>p.Tyr1241Cys<br>*pending*<br>transmembrane domain D3S2 | VUS<br>(PM1, PP2)<br><br>MPC=2.48 | None | 8 m | F, A | Focal posterior bilateral EDs | NA / MID | Sz free: VGB | VGB | Paroxysmal dyskinesia |
| 11 | 2 y 1 m | c.3953A>G<br>p.Asn1318Ser<br>*de novo*<br>cytoplasmic linker D3S4/D3S5 | Likely pathogenic<br>(PS2, PM2, PP2)<br><br>MPC=2.04 | None | 3 m | F, GTC | Normal<br>Ictal: EDs on the P regions bilaterally | N / N | Sz reduction: CBZ, PER<br>No effect: PB, OXC, CLB, ZNS<br>Adverse effects: TPM (hyperthermia) | | Ataxia |
| 12 | 2 y 3 m | c.3956C>A<br>p.Ala1319Asp<br>*de novo*<br>cytoplasmic linker D3S4/D3S5 | Likely pathogenic<br>(PS2, PM2, PP2)<br><br>MPC=2.69 | None | 2 m | C, T, GTC | Normal<br>Ictal EEG: parasagittal EDs, F rhythmic delta | N / MID | Sz reduction: PHT<br>No effect: VPA, PB | | Ataxia,<br>Tremor, language delay |

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| 13 | 7 y 11 m | c.3967G>A p.Ala1323Thr, de novo cytoplasmic domain D3S4/D3S5 | Likely pathogenic (PS2, PM2, PP2) MPC=2.22 | None | 5 m | FS, GTC | Normal | N / N | Sz free: VPA | VPA | |
| 14 | | c.4391T>C p.Ile1464Thr *paternal* inactivation gate | Likely pathogenic (PM1, PM2, PP1, PP2) MPC=2.65 | See fig. 1 | 7 m | A, GTC, SE | Diffuse abnormal | NA / MID | Sz reduction: TPM, VPA | | |
| 15 | 10 y 6 m | c.4423G>A p.Gly1475Arg, *paternal*, inactivation gate | Likely pathogenic (PS1, PM2, PP1, PP2) MPC=1.54 | See fig. 1 | 9 m | F to GTC, AA | Slowing over the temporal region (3y), then normal (9y) | NA / MID | Sz free: LTG Sz reduction: OXC, CBZ No effect: LEV | LTG | Hypotonia, ataxia, autistic features |
| 16 | 9 m | c.4423G>A p.Gly1475Arg *de novo,* inactivation | Pathogenic (PS1, PS2, PP2) MPC=1.54 | None | 4 m | F, T | Ictal: Slowing and EDs on both hemispheres, mainly | NA / MID | Sz free: PHT No effect: LEV | PHT | Hypotonia, ataxia |

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| | | gate | | | | | temporal regions | | | | |
| 17 | 9 y 9 m | c.4423G>A p.Gly1475Arg *de novo* *Blood mosaicism 10%* inactivation gate | Pathogenic (PS1, PS2, PP2) MPC=1.54 | None | 11 m | GTC, F | Normal | N / N | Sz free: CBZ Sz reduction: VPA | CBZ | |
| 18 | 8 y | c.4447 G>A, p.Glu1483Lys *de novo* inactivation gate | Pathogenic (PS1, PS2, PS3, PM2, PP2) MPC=2.13 | None | 11 m | F, GTC | Normal background, small Spikes / theta pointu in the central regions | N / N | SZ free: VPA, CBZ, currently no AED | | Clumsy, shy, speech delay |
| 19 | 2 y | c.4585A>G p.Met1529Val *de novo* transmembrane D4S1 | Likely pathogenic (PS2, PM1, PM2, PP2) MPC=1.2 | None | 4 m | GTC | Right posterior slow activity during sleep Ictal: generalized spike discharges | N / N | No effect: LEV, PB, VPA | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 11 y | c.4764C>G p.Phe1588Leu *de novo* transmembrane D4S3 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4 | None | 3.5 m | GTC, AA, T, F | Normal | DD / MID | Sz free: CBZ | CBZ | Autism, language delay |
| 21 | 13 y | c.4764C>A p.Phe1588Leu *de novo,* transmembrane D4S3 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4 | None | 5 m | F, GTC | NA | N / MID | Sz reduction: PB, CBZ | | Obesity |
| 22 | 2 y 8 m | c.4840A>G p.Thr1614Ala *de novo* extracellular domain D4S3/D4S4 | Likely pathogenic (PS2, PM2, PP2) MPC=2.17 | None | 4.5 m | AA, GTC, M, A | Generalized SW | N / MID | Sz reduction: VPA, OXC | | |
| 23 | 7 y 5 m | c.4850G>C p.Arg1617Gln *de novo* transmembrane D4S4 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4 | None | 5 m | GTC | Normal | DD / MD | Sz free: LTG | LTG | Autism |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 9 y | c.4850G>A p.Arg1617Gln *de novo* transmembrane D4S4 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4 | Paternal first cousin with microcephaly | 5 m | GTC, M, A | NA | DD / MID | | | Language delay, paroxysmal dyskinesia |
| 25 | 24 y | c.4850G>A p.Arg1617Gln *de novo* transmembrane D4S4 | Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC=2.4 | None | NA | GTC, F | NA | MID / MID | Sz free: CBZ, VPA | | Extra-pyramidal signs |
| 26 | 7 y | c.4949 C>T p.Ala1650Val *de novo* cytoplasmic D4S4/D4S5 | Pathogenic (PS1, PS2, PM2, PP2) MPC=2.26 | None | 11 m | M, A, T, S, AA | Generalized EDs | NA / MID | Sz free: CLB, RUF, KDSz adverse effects: LEV (increased frequency), ZNS (new sz type), VPA (neurodevelopmental regression) | | Hypotonia, supraventricular tachycardia |
| 27 | 9 y | c.4961T>A | Likely | None | 2 y 8 m | Ab | Generalized 3 | NA / MID | No effect: | | Ataxia |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | | p.Ile1654Asn<br>*unknown*<br>transmembrane domain D4S5 | pathogenic (PM1, PM2, PP2)<br><br>MPC=2.92 | | | | Hz SW. Spikes occipital right and left | | VPA | | |
| 28 | 4 y | c.5273T>C<br>p.Val1758Ala<br>*de novo*<br>transmembrane D4S6 | Likely pathogenic (PS2, PM1, PM2, PP2)<br><br>MPC=2.46 | None | 2 y | Ab, FS | Mild BG slowing, irregular generalized EDs, OIRDA<br>Ictal: irregularly generalized, 3Hz SW followed by rhythmic diffuse delta activity (clinically: arrest of ongoing activity) | N / N | Sz free: ETX<br>No effect: LEV | ETX | |
| 29 | 9 y | c.5311G>A<br>p.Val1771Ile<br>*de novo* | Likely pathogenic (PS2, PM2, PP2) | None | 6 m | GTC, T | Normal | N / N | Sz reduction: CBZ | | |

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| | | C-terminal | MPC=2.02 | | | | | | | | |
| 30 | 6 y | c.5458C>T p.Arg1820* *de novo* C-terminal | Pathogenic (PVS1, PS1, PM2, PP2) | None | 3-4 m | M in hands and fingers | Trains of SW, right CP. Irregular delta activity on the left side | NA / MID | Sz reduction: LTG | | Ataxia, language delay |
| 31 | 15 y | c.5497G>C p.Asp1833His *unknown* C-terminal | Likely pathogenic (PM1, PM2, PP2) MPC=2.44 | None | 6 m | T | EDs over the posterior lobe | N / N | Sz free: PB No effect: LEV | PB | Reflux |
| 32 | 3 y | c.5597G>A, p.Arg1866Gln, *de novo* C-terminal | Likely pathogenic (PS2, PM2, PP2) MPC=2.39 | None | 9 m | F, A, AA | EDs, mid CP | N / Mild learning disability | Sz free: CBD No effect: CBZ, CLB, LEV, TPM, VGB, VPA, ST Adverse effects: CBZ (drowsiness and severe cognitive disturbance) | CBD | |

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| 33 | | c.5615G>A p.Arg1872Gln *paternal (mosaic)* cytoplasmic c-terminal | Likely pathogenic (PS1, PM2, PP1, PP2) MPC=2.39 | See fig. 1 | 6 wk | F, clusters of GTC, SE | Normal | N / N | Sz free: CBZ + LEV | | Learning difficulties |
| 34 | 14 y | c.5630A>G, p.Asn1877Ser, pending cytoplasmic c-terminal | Likely pathogenic (PS1, PM2, PP2) MPC=2.04 | None | 5 m | F clustering | Normal | N / N | Sz free: LTG | LTG | Language delay |
| 35 | 4 y 4 m | c.5630A>G Asn1877Ser *paternal* cytoplasmic c-terminal | Likely pathogenic (PS1, PM2, PP1, PP2) MPC=2.04 | See fig. 1 | 6 m | | Normal | N / MID | Sz free: LTG, VPA | | |
| 36 | 4 y 6 m | c.5630A>G p.Asn1877Ser *unknown* cytoplasmic c-terminal | Likely pathogenic (PS1, PM2, PP2) MPC=2.04 | None | 7 m | GTC, T, AA | Sporadic EDs, FC bilateral > right | N/ MID | Sz reduction: VPA, PB, TPM | | Ataxia, clumsiness |

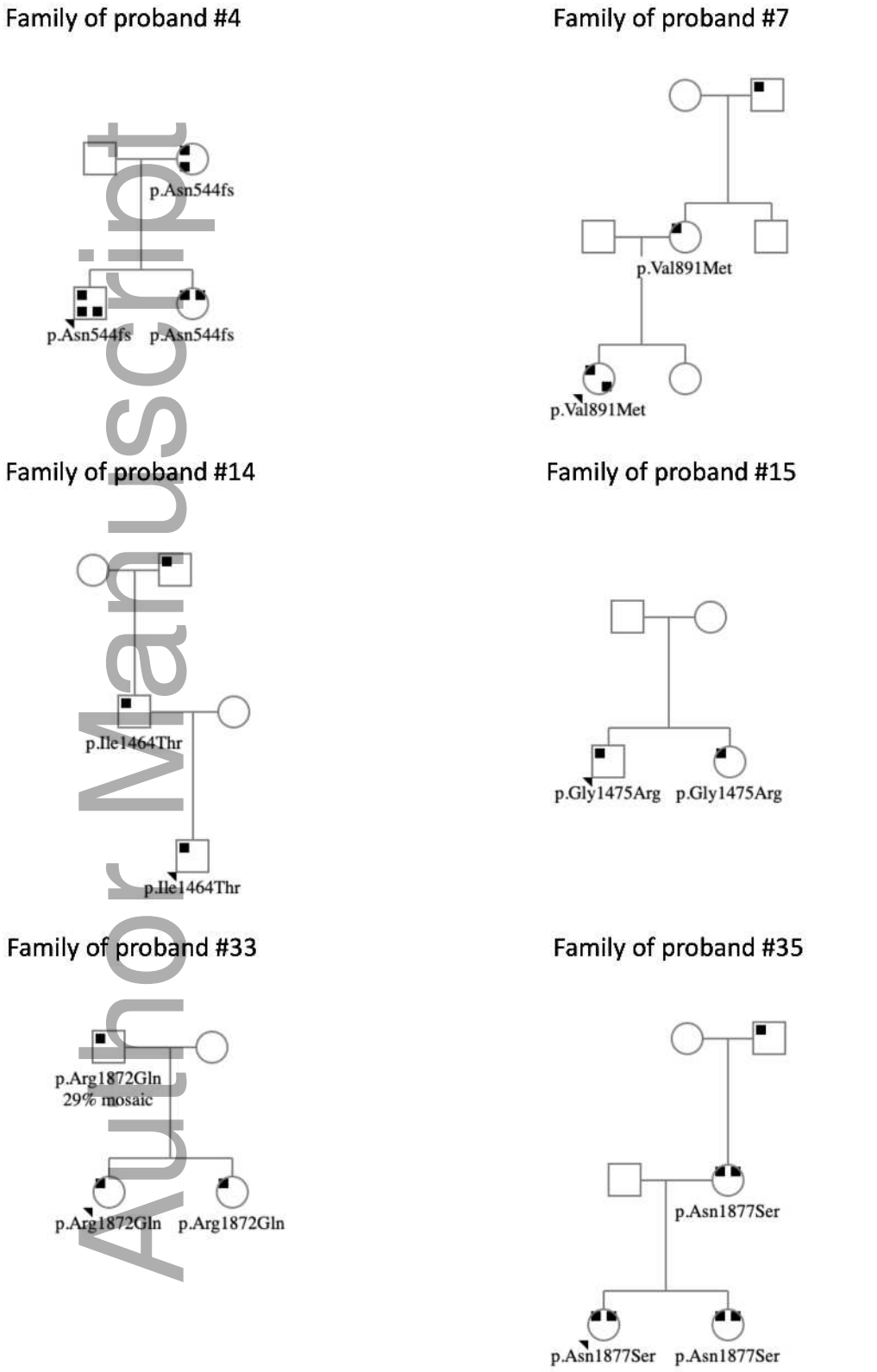
Abbreviations: A: Atonic, AA: Atypical Absences, Ab: Absences, ADHD: Attention Deficit Hyperactivity Disorder, AMD: amantadine, BG: Background, CAE: Childhood Absence Epilepsy, CBZ: Carbamazepine, CLB: Clobazam, CLZ: Clonazepam, D:Domain, DD: Developmental Delay, ED: Epileptiform Discharges, FS: Febrile seizures, F: Focal, GTC: Generalized Tonic-Clonic, KD: Ketogenic diet, LAC: Lacosamide, LEV: Levetiracetam, LTG: Lamotrigine, , LZP: Lorazepam, MD: Moderate Intellectual Disability, MID: Mild Intellectual Disability, m: months, M: Myoclonic, NA: Not available, N: Normal, O: Occipital, OXC: Oxcarbazepine, P: Parietal, PER: Perampanel, PHT: Phenytoin, RFM: Rufinamide, S:SE: Status Epilepticus, ST: Stiripentol, S: Spasms, SW: Spike and Wave complexes, sz: Seizures, T: Tonic, TPM: Topiramate, VGB: Vigabatrin, VPA: Valproic Acid, y: years, ZNS: Zonisamide

**Figure legends:**

Figure 1. Pedigrees of *SCN8A*-related epilepsies showing segregation of the variant with the phenotype

Figure 2. The spectrum of *SCN8A*-related epilepsy

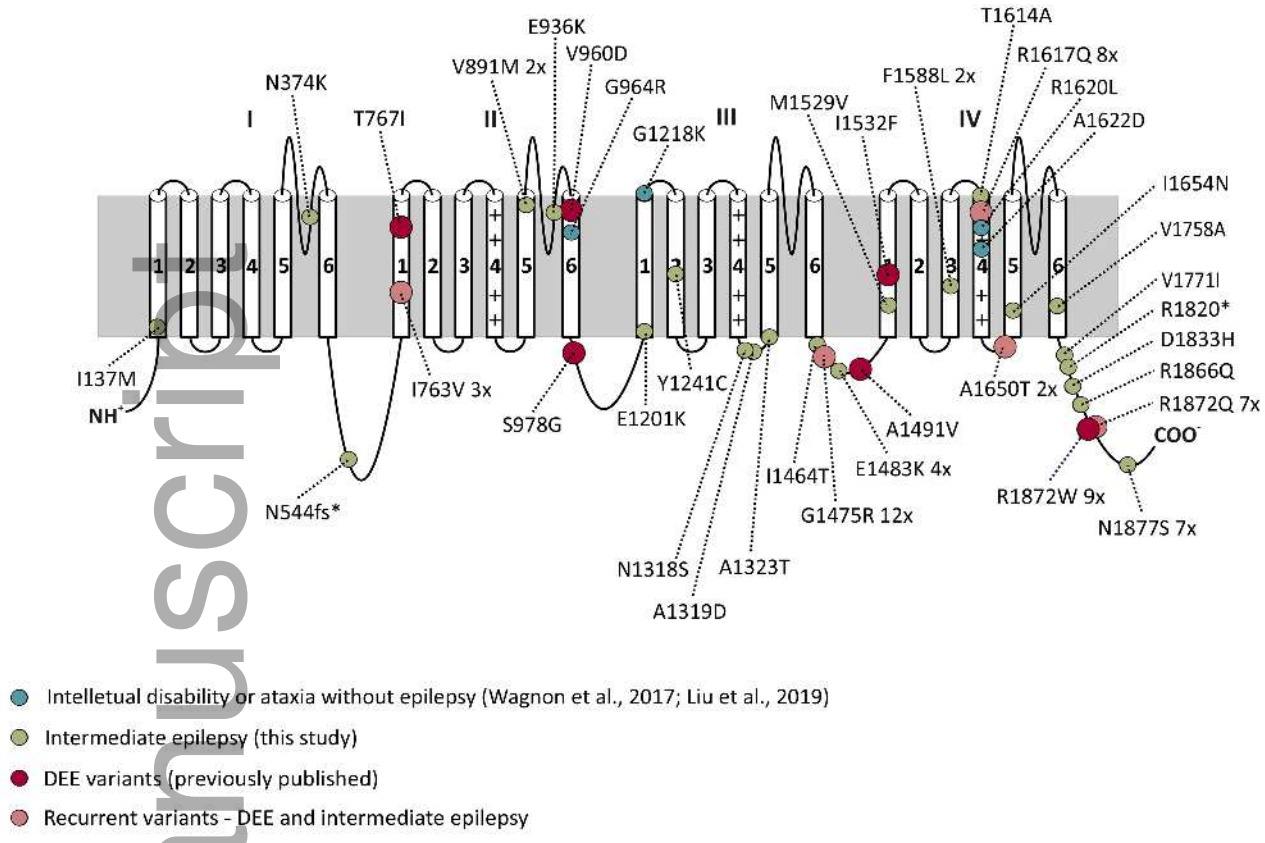
Figure 3. A partial display of published variants, with the recurrent DEE and known LOF variants, as well as those identified in this study






epi_14705_f1.tiff


epi_14705_f2.tiff




epi_14705_f3.tif